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2. Patent application number
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0300001.5

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Neil Polwart
2 Kingsfield
Linlithgow
West Lothian
EH49 7SJ

Patents ADP number *(if you know it)*

08535700001

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

4. Title of the invention

Improved Surface Plasmon Resonance Sensor

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Kennedys Group
Floor 5, Queens House
29 St Vincent Place
Glasgow
G1 2DT

Patents ADP number *(if you know it)*

08036458002

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

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Claim *(s)*	
Abstract	
Drawing *(s)*	3 TS

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Request for preliminary examination and search *(Patents Form 9/77)*	
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11. I/We request the grant of a patent on the basis of this application.

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KENNEDYS

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David Fulton 0141 226 6826

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Improved Surface Plasmon Resonance Sensor

This invention relates to a Surface Plasmon Resonance Sensor. In particular it relates to an improved design of Surface Plasmon Resonance Sensor that is compact, mobile and cost effective thus making it ideal for field applications.

The phenomenon of Surface Plasmon Resonance (SPR) is well known to those skilled in the art having being first demonstrated over twenty five years ago. Surface Plasmon Resonance is a charge-density oscillation that may exist at the interface of two media that exhibit dielectric constants of opposite signs, for example a metal and a dielectric.

Surface Plasmon Resonance sensors described in the Prior art generally comprise an optical system, a transducing medium that generally combines the optical system and the relevant chemical or biochemical domains, and an electronic system that supports the optoelectronic components of the sensor and allows for the required data processing. The devices come in three main configurations namely:

(1) Prism coupler based systems;

(2) Grating coupler based systems; or

(3) Optical waveguide based systems.

A typical prism coupler based system 1 is presented schematically in Figure 1. This system is generally accepted as being the best suited for sensing and therefore has become the most widely employed system in the art. In this configuration a light wave 2 passes through a first element of an optical system 3 before passing into a prism 4. Thereafter, the light wave 2 experiences total internal reflection at the interface between the prism 4 and a thin metal layer 5 (typically of a thickness of around 50 nm). The light wave 2 then passes through a second element of the optical system 6 that acts to manipulate the light wave 2 such that it becomes incident on a detector 7.

The Surface Plasmon Resonance sensor 1 is an ideal medium for analysing samples that become attached to the metal layer 5. SPR is a phenomenon that occurs when light incident upon the metallic layer 5 provides an absorption energy capable of vibrationally exciting the packets of electrons (or plasmons) located on the surface of the metal layer 5. As such the energy required to achieve SPR is highly dependent upon the dielectric constant of the species at the surface of the metal, the wavelength of the light wave 2 and the angle of incidence of the light wave 2.

As is known in the art the use of a particular monochromatic light source of a known wavelength incident at variable angles, or across a range of known angles, allows a reference Reflectance Angle versus Intensity

data to be recorded. The presence of any foreign bodies that become attached to the surface of the metal layer 5 then act to change the value of the dielectric constant experienced by the light wave 2 at the surface of the metal layer 5. As such the presence of these foreign bodies can be easily detected and thereafter quantified by monitoring the profile of the Reflectance Angle versus Intensity curves.

The systems described in the Prior Art are not easily miniaturised and as such are not easily adapted to be used as field based instruments. Therefore, a user requires to take a sample that then needs to be taken to the laboratory for testing which can lead to significant delays in obtaining results. Such delays can be fatal when the instruments are employed as biosensors to detect particular pathogens.

It is an object of an aspect of the present invention to provide a Surface Plasmon Resonance Sensor that is compact, mobile and cost effective thus making it ideal for the field detection of pathogens in, for example, water systems.

According to a first aspect of the present invention there is provided a cartridge for use in a sensor, the cartridge comprises an optical element having a first surface for the entry of a light beam incident on the optical element and a mounting member for supporting a sensing agent located on a second surface of the optical element wherein the first surface includes a first means for directing the light beam incident on the optical element towards the second surface at an angle of incidence to the second surface that results in

substantially total internal reflection of the light beam at a boundary of the mounting member and the second surface.

Most preferably the optical element further comprises a third surface for the exit of the light beam from the optical element wherein the third surface includes a second means for directing the light beam.

Preferably the optical element comprises a material having a first dielectric constant while the mounting member comprises a material having a second dielectric constant wherein the second dielectric constant is of an opposite sign to that of the first dielectric constant.

Most preferably the first means for directing the light beam comprises a focusing element for focusing the light beam to a line on the boundary of the mounting member and the second surface.

Preferably the second means for directing the light beam comprises a defocusing element.

Preferably the mounting member comprises a metal.

Preferably the optical element comprises an injection moulded plastic material.

Most preferably the sensing element comprises an antibody suitable of binding one or more pathogens.

Preferably the pathogen suitable for being bound to the antibody comprises a bacterium selected from the group comprising Legionella, Escherichia coli, Salmonella,

Bacillus Anthracis, Yersinia Pestis, Lysteria, Cryptosporidium, Variola virus, Picomaviridae Apthovirus, Filoviruses, any plasticiser, steroid, medicinal drug or illicit substance or any other known fluid borne bacterium.

Preferably a protein substrate and a ligand binds the biotinylated antibody to the metal.

Preferably the protein substrate comprises biotin.

Preferably the ligand comprises a protein selected from the group comprising avidin, strepavidin and neutravidin.

According to a second aspect of the present invention there is provided a Surface Plasmon Resonance sensor comprising a light source for generating a light beam, a cartridge according to the first aspect of the present invention, a channel capable of containing a fluid sample to be tested and a light beam detection means wherein the cartridge allows for the miniaturisation of the sensor.

Most preferably the light source comprises a diode laser.

Preferably the channel locates on the second surface of the cartridge such that the fluid sample contained within the cartridge makes physical contact with the mounting member.

Preferably the light beam detection means comprises a detector and a data processing means.

According to a third aspect of the present invention there is provided a method for the field detection of one

or more pathogens that employs a Surface Plasmon Resonance sensor in accordance with the second aspect of the present invention comprising the steps of:

1) Selecting the appropriate cartridge for the one or more pathogens to be tested for;
2) Calibrating the Surface Plasmon Resonance sensor; and
3) Testing of a fluid sample for the presence of one or more of the pathogens;

Preferably the selection of the appropriate cartridge comprises locating the cartridge with one or more appropriate antibodies within the Surface Plasmon Resonance sensor.

Preferably calibrating the Surface Plasmon Resonance sensor comprises:

1) Irradiating the mounting member with the light beam in the absence of the fluid sample; and
2) Detecting the light beam and storing the data as a reference signal;

Preferably testing of the fluid sample for the presence of one or more pathogens comprises:

1) Locating the fluid sample with respect to a channel;
2) Connecting the channel to the disposable cartridge;
3) Irradiating the fluid sample with the light beam;
4) Detecting the light beam and storing the data as a sample signal; and
5) Analysing the test results by comparing the sample signal to the reference signal.

Embodiments of the invention will now be described, by way of example only, with reference to the accompanying drawings, in which:

Figure 1 present a prism coupler based Surface Plasmon Resonance sensor as described in the Prior Art;

Figure 2 present a disposable cartridge based Surface Plasmon Resonance sensor in accordance with an aspect of the present invention;

Figure 3 present a schematic representation of the Surface Plasmon Resonance sensor of Figure 2; and

Figure 4 present a schematic representation of a binding method employed by the Surface Plasmon Resonance sensor of Figure 2; and

Figure 5 presents typical Angle versus Intensity curves as may be obtained by the Surface Plasmon Resonance sensor.

Figures 2 and 3 present a disposable cartridge based Surface Plasmon Resonance sensor 8 in accordance with an aspect of the present invention. The sensor can be seen to comprise a diode laser 9, a disposable cartridge 10 and a charge coupled device (CCD) detector 11 that is connected to a data processing unit 12.

The disposable cartridge 10 comprises a shaped entrance surface 13, a shaped exit surface 14 and a gold strip 15 that is attached to a third side of the disposable cartridge 16. A channel 17 is employed to enclose the gold strip so providing a means for containing or passing a fluid sample across the surface of the gold strip 15.

The disposable cartridge 10 can be removed from the channel so as to enable the cartridge 10 to be replaced as required.

In order that the cartridge 10 be correctly aligned to the diode laser 9, the CCD detector 11 and located correctly with the channel 17, the channel 17 may further comprise either male of female members (not shown) that interact with female or male members, respectively, located on the surface of the cartridge 10.

In order for the Surface Plasmon Resonance sensor 8 to operate correctly there must be a means whereby the relevant pathogen 18 to be detected can attach to surface of the gold strip 15. There are several techniques known to those skilled in the art for binding pathogens 18 to a metal strip..

Figure 4 presents a schematic representation of a binding method suitable for use with the Surface Plasmon Resonance sensor 8. The first stage involves binding a suitable protein substrate 19, for example biotin, to the surface of the gold strip 15. Stage two involves attaching a ligand 20 to the protein substrate 19. A suitable ligand 20 for conjugating with biotin is avidin although steptavidin or neutravidin may also be employed. The third stage then involves the attachment of an antibody 21, appropriate for the relevant pathogen 18 to be tested for, to the ligand 20. This attachment is achieved by employing antibodies 21 that have been biotinylated 22.

When the gold strip 15 has been treated as described above the Surface Plasmon Resonance sensor 8 is ready for

use. The diode laser 9 provides the required light beam 23. The light beam 23 is focused to a line 24 on the gold strip 15 on passing through the shaped entrance surface 13. This provides a large area of interaction between the light beam 23 and the gold strip 15. Such an area of interaction allows a range of spatially resolved biotinylated antibodies 22 to be deposited on a single cartridge 10. The light beam 23 is then totally internally reflected so as to traverse through the shaped exit surface 14. This results in the light beam 23 being defocused such that the incident signal from each of the biotinylated antibodies 22 is spatially resolved across the whole area of the CCD detector 11. Data processing can then be carried out on the detected signal as appropriate.

Figure 5 presents a schematic Reflectance Angle versus Intensity curves that may typically be obtained by the Surface Plasmon Resonance sensor 8. The solid curve 25 corresponds to the case where no pathogen 18 is present in the fluid sample as indicated in Figure 5(a). However, Figure 5(b) shows the case when a pathogen 18 is present in the fluid sample, as represented by the broken curve 26. The pathogen 18 on becoming attached to the surface of the gold strip 15 alters the value of the dielectric constant experienced by the light beam 23 at the surface of the gold strip 15. As such the presence of the pathogen 18 alters the profile of the Angle versus Intensity curve, so permitting quick and easy detection of the presence of the pathogen 18.

The employment of the disposable cartridge 10 and a diode laser 9 light source provides the Surface Plasmon Resonance sensor 8 with significant inherent advantages

over those taught in the Prior Art. In the first instance these elements allow for the significant miniaturisation of the device such that the Surface Plasmon Resonance sensor 8 provides a compact, mobile and cost effective device for the field testing of the presence of a pathogen 18. The miniaturisation of the device has the added advantage that it increases the sensitivity of the sensor since all of the functionalised area of the gold strip 15 can be contained within the focused line 24 area of the incident light beam 23.

Having the focusing and defocusing elements incorporated directly within the disposable cartridge 10 removes the time consuming alignment requirements associated with the optical systems 3 and 6 of the Prior Art sensors. In addition by employing an injection moulding technique allows for the low cost fabrication of the disposable cartridge 10. Such a technique therefore makes it cost effective to remove and dispose of the cartridge 10 after use and simply replace it with a new cartridge 10 as required. The use of these disposable cartridges 10 significantly reduces the time consuming cleaning requirements associated with the sensors described in the Prior Art.

The Surface Plasmon Resonance sensor 8 described herein is particularly suitable for the detection of the bacteria Legionella in water samples obtained from industrial or recreational sources. This is of particular importance in evaluating and controlling the risk to public health presented by the often-fatal condition Legionnaires disease and the less serious but far more common condition of Pontiac Fever. Existing techniques are either very slow or too labour insensitive

to meet market demands as these generally require qualified microbiologists to perform testing at specialist laboratories.

The availability of the focused line 24 interaction area on the gold strip 15 allows for the functionalisation of the interaction area for different antibodies that are sensitive to different forms of the Legionella bacteria. Thus this apparatus provides for a sensor capable of simultaneously detecting and discriminating between Legionella pnuemophilla serogroup 1 and Legionella serogroups 2-15.

Although ideal for the detection of the bacteria Legionella it will be obvious to one skilled in the art that the surface Plasmon Resonance sensor may be easily adapted for use in the detection of alternative species e.g. Escherichia Coli, Salmonella, Bacillus Anthracis, Yersinia Pestis, Lysteria, Cryptosporidium, Variola virus, Picomaviridae Apthovirus, Filoviruses, any plasticiser, steroid, medicinal drug or illicit substance or any other known fluid borne pathogen.

In addition to the use for water quality monitoring as described above it would be obvious to one skilled in the art that the Surface Plasmon Resonance sensor 8 is also ideal for use in healthcare, especially for use as a point of care diagnostic.

Aspects of the present invention described above offer significant advantages over the Prior Art. In the first instance the Surface Plasmon Resonance sensor provides a compact, mobile and cost effective device for the field testing of the presence of a pathogen. The device is

ideal for the expeditious detection and identification of a range of pathogens. Further, the incorporation of the focused line area provides a means for carrying out such a detection and identification process simultaneously for a number of different pathogens.

The foregoing description of the invention has been presented for purposes of illustration and description and is not intended to be exhaustive or to limit the invention to the precise form disclosed. The described embodiments were chosen and described in order to best explain the principles of the invention and its practical application to thereby enable others skilled in the art to best utilise the invention in various embodiments and with various modifications as are suited to the particular use contemplated. Therefore, further modifications or improvements may be incorporated without departing from the scope of the invention herein intended.

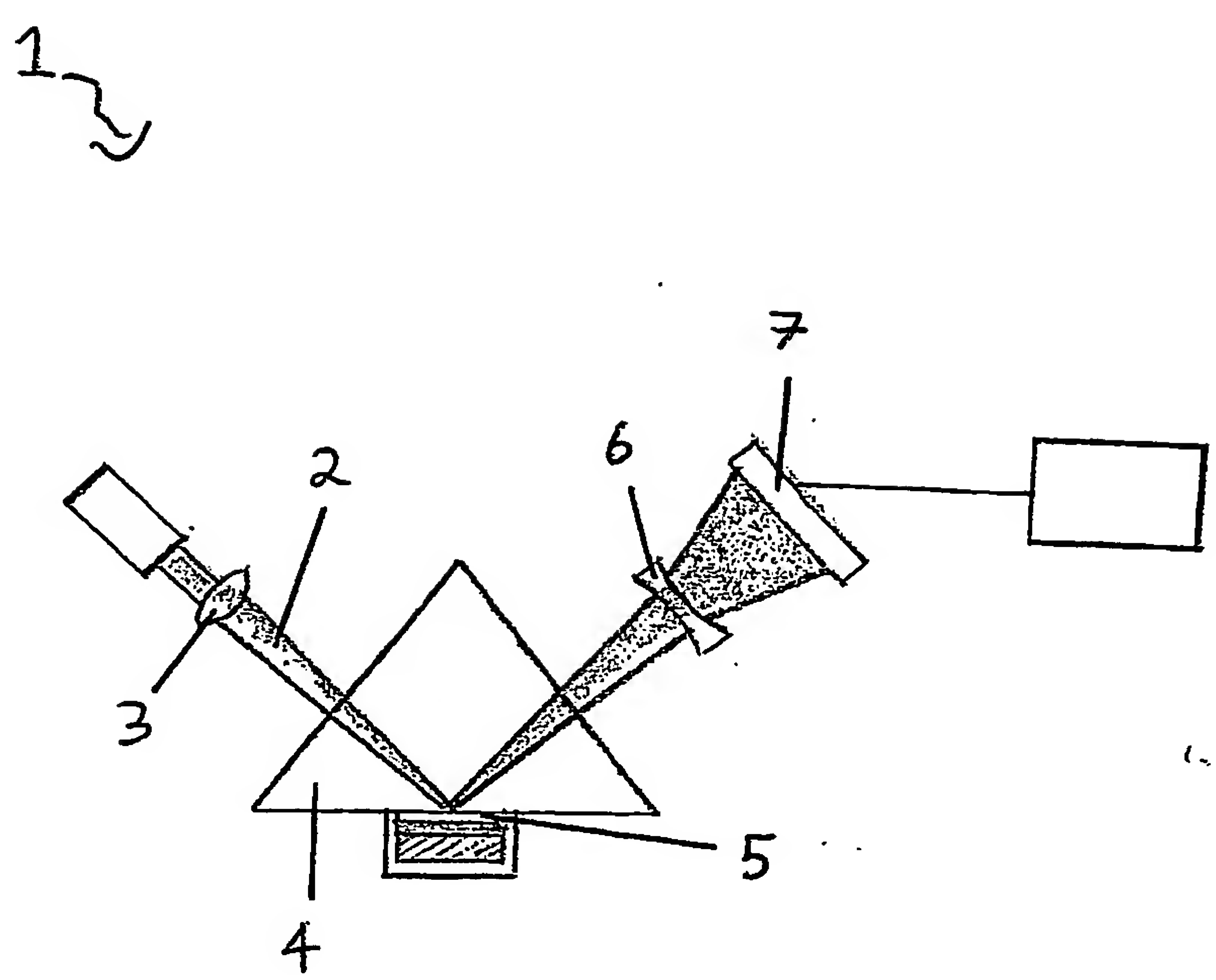

FIGURE 1

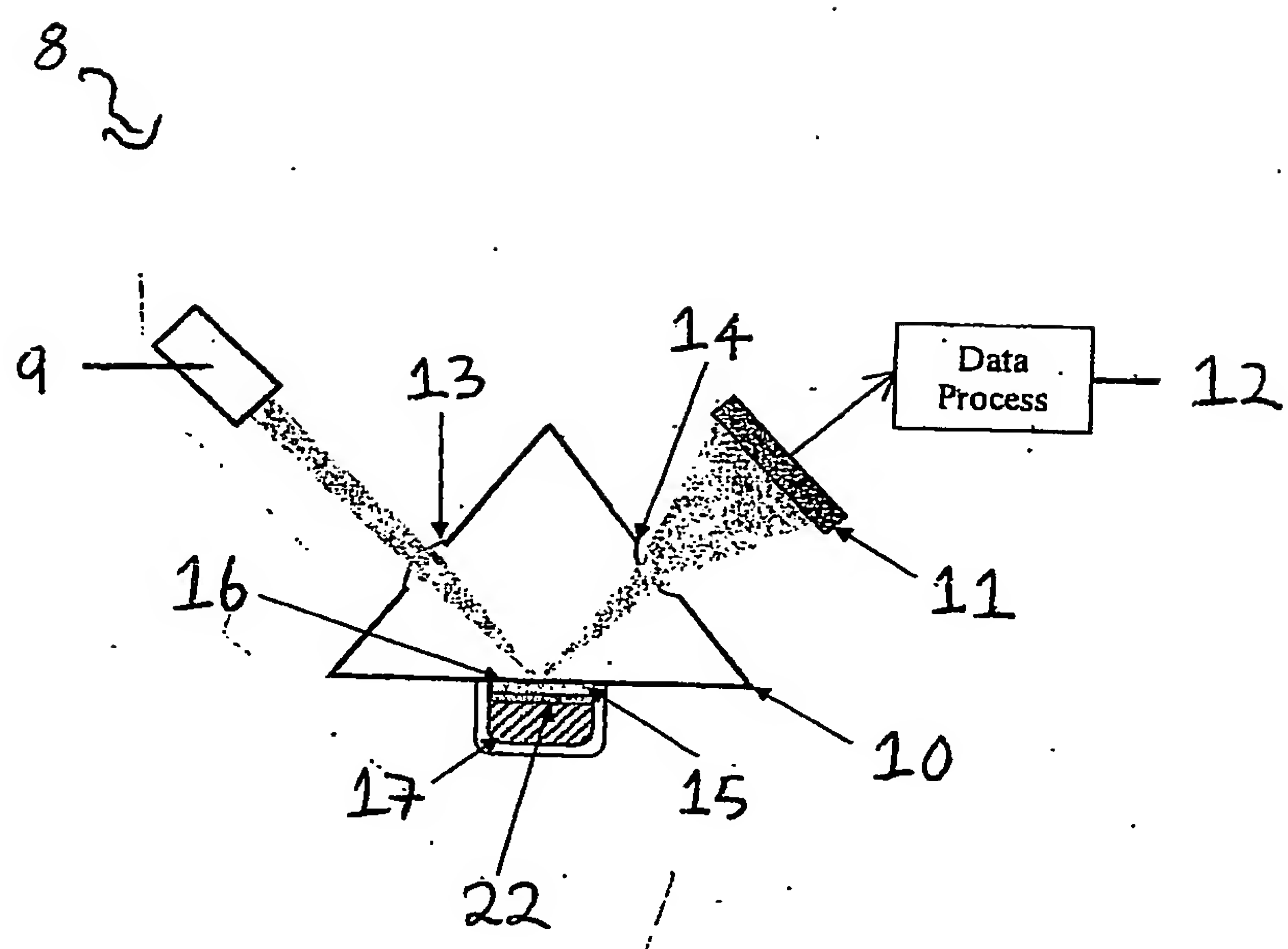

FIGURE 2

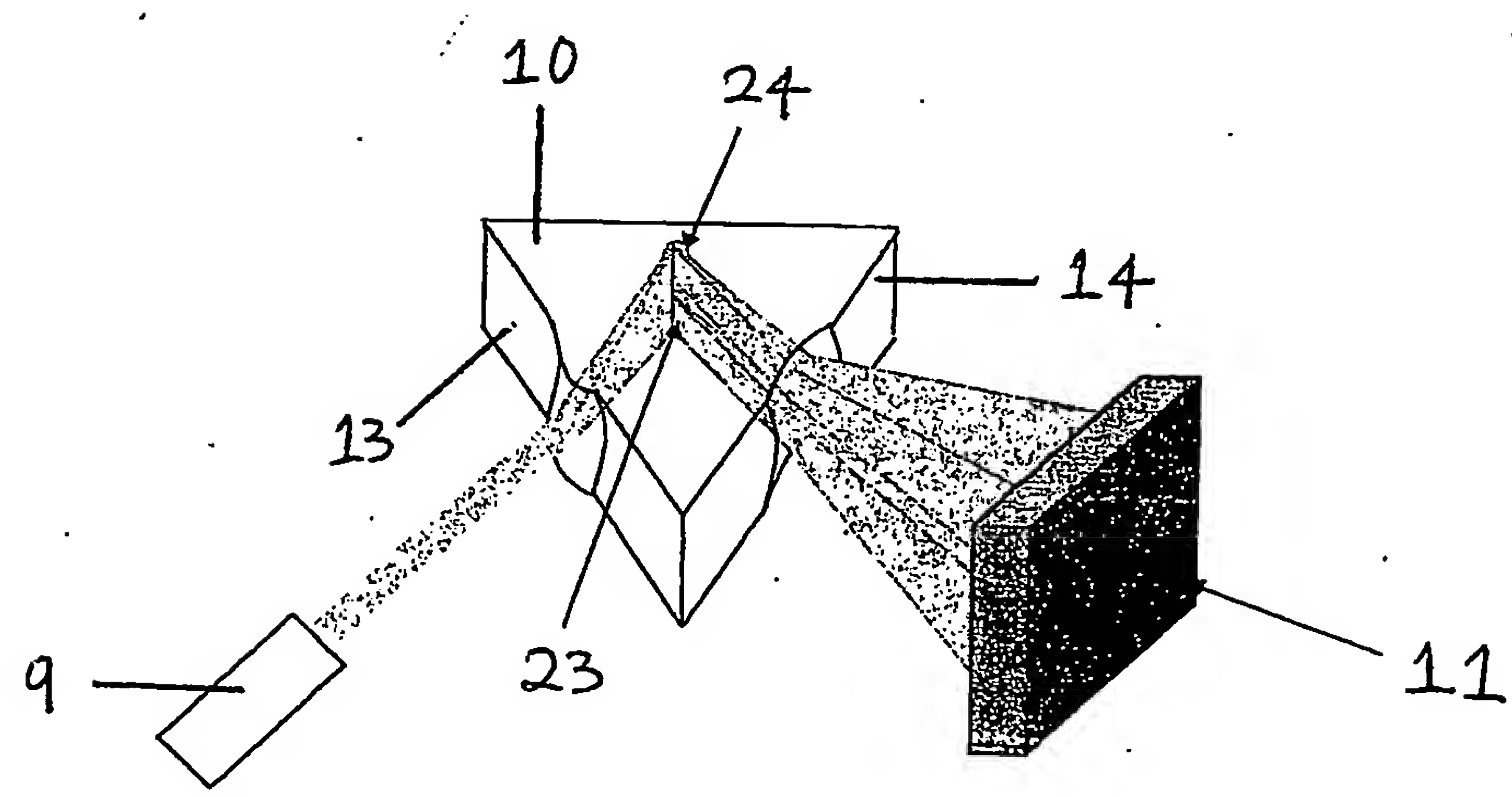

FIGURE 3

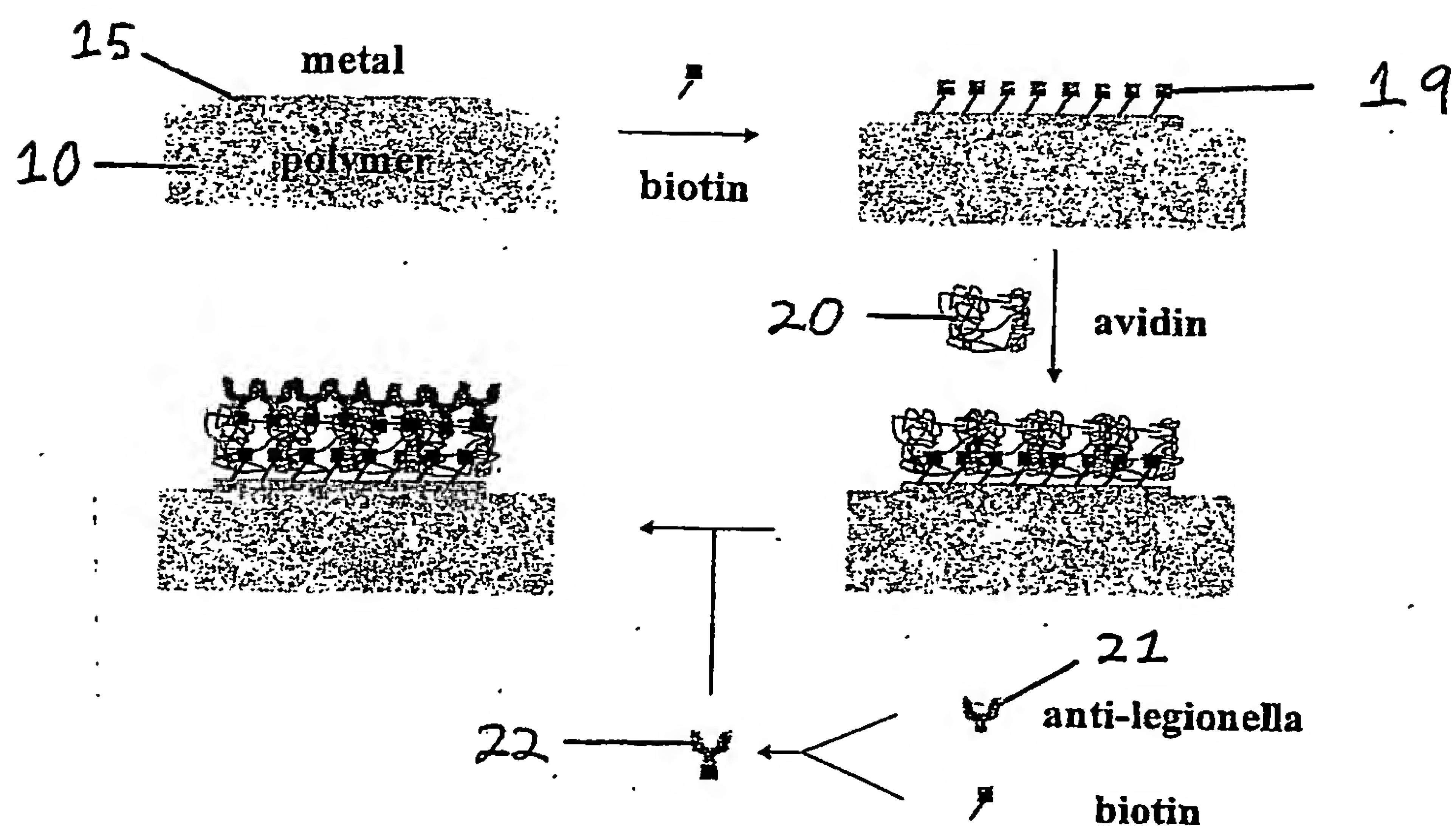

FIGURE 4

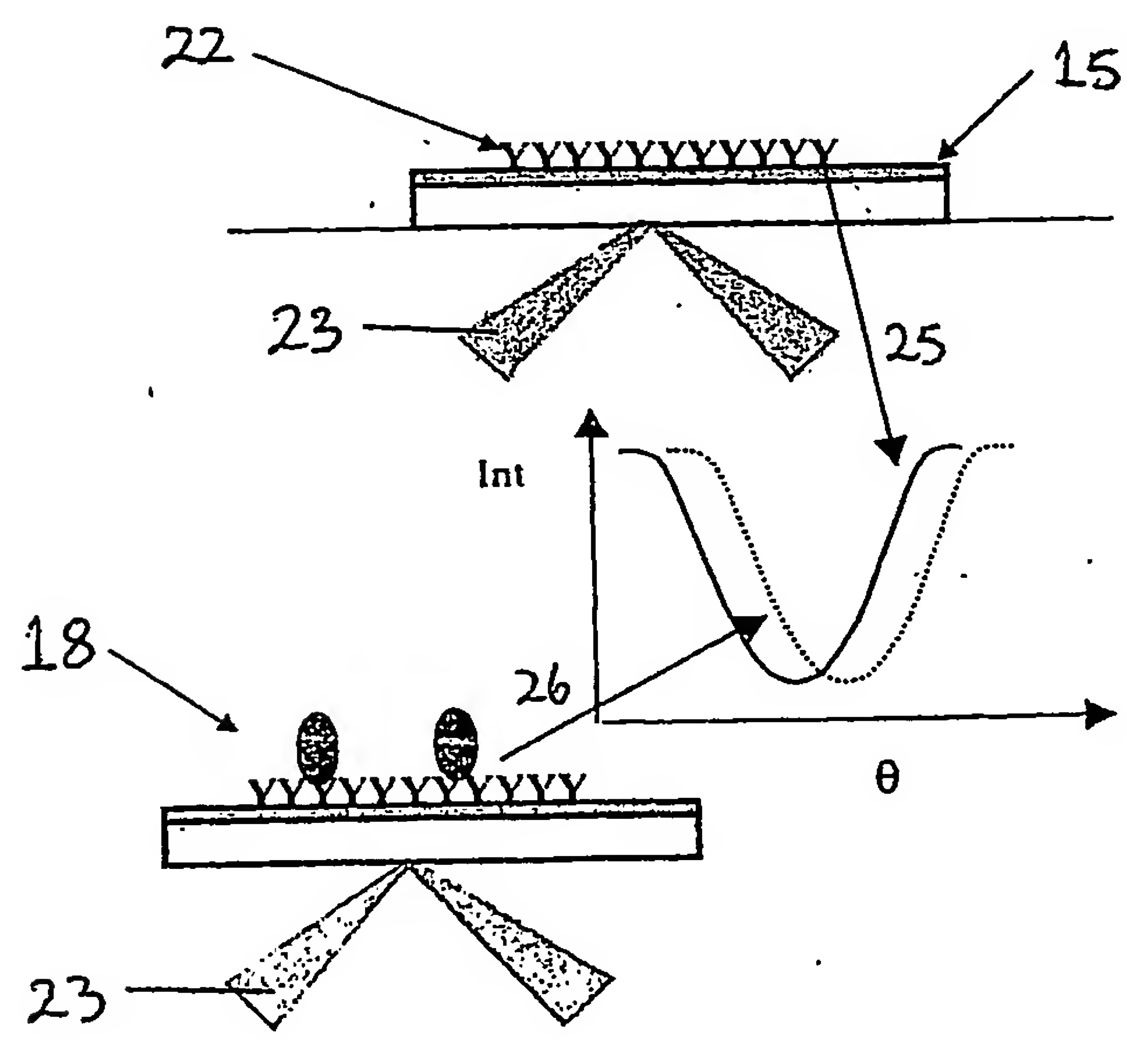

FIGURE 5

PCT Application

PCT/GB2003/005716